# $^{18}$F-AlF–Labeled Biomolecule Conjugates as Imaging Pharmaceuticals

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The need for receptor-targeted PET imaging pharmaceuticals led to the discovery and development of numerous radiolabeled peptides and proteins specific to receptors that are known to overexpress in certain tumors (Supplemental Table 1; supplemental materials are available at http://jnm.snmjournals.org) (*1*). Some target-specific biomolecules known to have high specificity and affinity for receptors associated with tumors and other pathologic conditions are folate, peptides (e.g., gastrin-releasing peptide, Arg-Gly-Asp, and somatostatin), and proteins (Affibody molecules [Affibody AB], antibodies, and antibody fragments) (Supplemental Table 2). Various metallic (e.g., $^{64}$Cu, $^{68}$Ga, and $^{89}$Zr) and nonmetallic ($^{124}$I) radionuclide-labeled biomolecules have been used in preclinical and clinical environments. Because of the desirable physical and radiochemical properties of the $^{18}$F radionuclide and the success of $^{18}$F-FDG in the clinic, there is tremendous interest in the development of $^{18}$F-labeled biomolecules—that is, peptides and proteins—as PET imaging pharmaceuticals.

Commonly, direct $^{18}$F labeling of biomolecules via carbon–fluorine bond formation is not feasible because the substrate in the reaction may not be able to handle harsh conditions. Three methodologies were developed for $^{18}$F labeling of peptides and proteins (*2*). The first was reaction of biomolecules with $^{18}$F-labeled prosthetic groups. The second was functionalization of a biomolecule via either a silicon or a boron acceptor group for $^{18}$F labeling by a displacement-and-isotope-exchange reaction or by a chelating group for $^{18}$F-AlF labeling. The third was use of click chemistry, which involves Cu(I)-mediated reaction of a functionalized peptide with a $^{18}$F-prosthetic group.

McBride et al. (*3*) discovered a novel methodology for $^{18}$F labeling of chelating agent–biomolecule conjugates based on an unusually strong fluoro bond between $Al^{3+}$ and fluoride (bond dissociation energy, 675 kJ/mol) and its tendency to form thermodynamically stable (*4*) and kinetically inert (*5,6*) metal chelates with polyaminocarboxylates, such as NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid). The hexadentate NOTA ligand or its derivatives are better suited for $AlF^{2+}$ labeling than is octadentate DOTA (1,4,7,10-tetraazacyclododecane–1,4,7,10-tetraacetic acid) because, first, the extra donor atoms in DOTA compete with fluoride coordination with $Al^{3+}$, since fully coordinated aluminum requires only 6 donor atoms (*7*), and second, the NOTA cavity is a better fit for the small $Al^{3+}$ cation than is the DOTA cavity. The $^{18}$F-AlF labeling procedure is fast and simple and does not require extra dry solvents or expensive, sophisticated automated synthesis modules. In a typical 1-pot, 1-step reaction, a mixture of $^{18}F^-$ with $AlCl_3$ solution at pH 4 (0.1 M acetate buffer) and chelating agent–peptide conjugate or a lyophilized formulation kit is incubated at 100°C for 15–20 min followed by reversed-phase high-performance liquid chromatography or cartridge purification. Sometimes, organic solvents and high reaction temperatures are used to improve the labeling yield. Despite the requirement of a cyclotron for $^{18}$F production, the $^{18}$F-AlF is a convenient substitute for $^{68}$Ga because of the shorter half-life and nonideal energies of $^{68}$Ga and the long lead time for availability and high cost of $^{68}$Ge/$^{68}$Ga generators. A comprehensive review of the $^{18}$F-AlF labeling of biomolecules and their preclinical and clinical evaluations was published recently (*7*).

Since the discovery of the $^{18}$F-AlF labeling technique in 2009, remarkable progress has been made. Numerous $^{18}$F-AlF–labeled target-specific biomolecules have been labeled with improved yields, formulation kits have been prepared, and $^{18}$F-AlF–labeled biomolecules have been evaluated in preclinical and clinical settings. The tests in the preclinical environment have included in vitro cell binding, serum stability, biodistribution, and PET imaging in mouse-xenograft models (Supplemental Table 2 (*8–26*)). Data from preclinical studies have suggested that these radiolabeled biomolecules remain intact during absorption, distribution, and elimination via a renal route or some time-combination of renal and hepatobiliary routes. The materials clear from blood rapidly (i.e., >90% cleared in 60 min).

$^{18}$F-alfatide I and $^{18}$F-alfatide II (Supplemental Fig. 1) were introduced into the clinic and showed feasibility in target-specific imaging of $\alpha_v\beta_3$ integrin expression in lung cancer patients, detection of metastasis in lymph nodes of differentiated thyroid cancer, and brain cancer. For example, 2 phase I clinical studies using $^{18}$F-alfatide I and $^{18}$F-FDG were conducted for detection of lung cancer in 9 patients and for detection of lymph node metastasis in differentiated thyroid cancer in 20 patients (*27,28*). All tumors were identified, with an $SUV_{mean}$ of 2.90 ± 0.10 in the lung cancer study (Supplemental Fig. 2) (*27*). Most differentiated thyroid cancer lymph node metastases showed abnormal uptake of $^{18}$F-alfatide I in the later study (*28*). However, alfatide I was a less effective diagnostic agent than $^{18}$F-FDG for lymph node metastasis. $^{18}$F-alfatide II was evaluated in 5 healthy volunteers and 9 patients with 20 brain metastasis tumors for safety, efficacy, and estimated absorbed dose. All brain lesions were visualized by $^{18}$F-alfatide II and showed a better tumor-to-background ratio than with $^{18}$F-FDG—that is, 18.9 ± 14.1 for $^{18}$F-alfatide II versus 1.5 ± 0.5 for $^{18}$F-FDG—demonstrating the value of $^{18}$F-alfatide II in detecting metastases as a biomarker of angiogenesis. Additionally, a pilot study

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involving 36 patients was conducted to verify the efficacy of $^{18}$F-alfatide II for detecting bone metastasis in humans, in comparison with $^{18}$F-FDG (*29*). $^{18}$F-alfatide II could detect bone metastasis lesions with good contrast and higher sensitivity than $^{18}$F-FDG—that is, a positive rate of 92% versus 77%.

Despite numerous preclinical studies with convincing data in animal models, only 2 tracers were translated into the clinic, with a limited number of trials (*7*). Possibly, additional probes are not meeting the researchers' expectations related to targetability and in vivo stability in human clinical trials. Moreover, preclinical studies do not always predict the expected outcome in the clinic. Lower than expected targetability and in vivo stability of the $^{18}$F-AlF–labeled biomolecules may be due to one or a combination of several factors, including, first, in vivo degradation of the biomolecule, because peptide and proteins are known to degrade by endogenous peptidase and proteases found in plasma and in most tissues (*30*), and second, the loss of $^{18}$F-AlF from the chelate.

Most of the chelate–biomolecule conjugates involve a NOTA or an HBED-CC (*N,N′*-bis [2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-*N,N′*-diacetic acid) chelating agent. The NOTA ligand forms a thermodynamically stable (log K being 17.9) and kinetically inert (only 1.5% dissociation in 1 M HCl) aluminum chelate (*4–6*). Additionally, spectroscopic studies and analysis of mouse blood and urine samples indicated that the Al(NOTA) chelate remains intact under physiologic conditions and the chelate does not undergo in vivo demetallation by transferrin (*6*).

However, one of the carboxylic acids in the NOTA chelating agent is conjugated with a biomolecule via hydroxysuccinimide or maleimide chemistry, converting NOTA to NO2A. Fluoride, therefore, coordinates with the sixth coordination site in aluminum. The thermodynamic stability and kinetic inertness of these AlF-chelated NO2A–biomolecule conjugates have unfortunately not been studied or reported. Supplemental Table 2 summarizes the limited serum stability data of $^{18}$F-AlF-labeled biomolecule conjugates, mostly at 1–2 h, and there is no information on the in vivo stability of these probes in preclinical models. Similarly, despite the high bond-dissociation energy of the aluminum–fluoro bond (675 kJ/mol) (*7*) and the reasonable first stepwise stability constant of AlF (log K = 6.40) (*7*), nothing is known about the in vitro or in vivo lability of AlF.

In summary, the procedure of $^{18}$F-AlF labeling of biomolecule conjugates is novel, fast, and simple; does not require expensive, sophisticated, or automated synthesis modules; and has tremendous potential. However, a design for novel, target-specific biomolecules with demonstrated long-term in vitro (buffered medium and serum) and in vivo stability, and a complete understanding of the physical and inorganic chemistry of AlF–biomolecule conjugates, are critical for future discovery and development of tracers for diagnosis of various disease targets.

## DISCLOSURE

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